

FIG. 4. The 'major extracellular proteases' of *B. subtilis* play a role in the degradation of extracellular, SsrA-tagged hIL-3. Western blot analysis of hIL-3 protein secreted by *B. subtilis* 168 harboring plasmid pLATIL3 (lane 1, 6) or pLATIL3TERM (lane 2, 7), and *B. subtilis* WB600 (a multiple protease negative strain) containing plasmid pLATIL3TERM and expressing either wild-type SsrA (lane 3, 8), no SsrA (lane 4, 9) or SsrA^{DD} (lane 5, 10). Culture supernatants of cells entering the stationary phase were collected, concentrated by TCA precipitation, analyzed by SDS-PAGE and immunoblotting with anti-hIL-3 antibody (lanes 1-5) or anti-Bs-SsrAtag antibody (lanes 6-10). SsrA-tagged hIL-3 (lanes 3, 5, 8, 10), run-off hIL-3 translation product (lane 4, and possibly also in lane 3 and 5, see text), and wild-type hIL-3 (lane 1) are indicated by the arrows (→). Protein bands with lower molecular weight that also react with anti-hIL-3 antibody are supposedly degradation products of hIL-3, SsrA-tagged hIL-3 or run-off hIL-3 translation product.

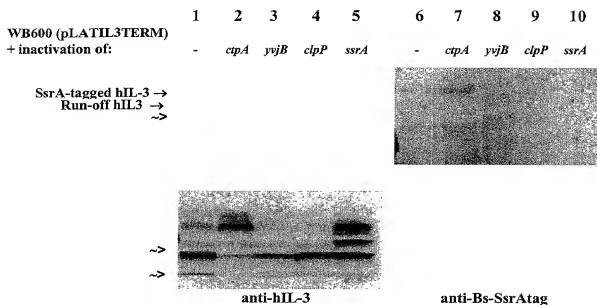


FIG. 5. *B. subtilis* CtpA has an additional role in the degradation of SsrA-tagged hIL-3. Western blot analysis of hIL-3 protein secreted by *B. subtilis* WB600 harboring plasmid (pLATIL3TERM) and carrying either no additional mutation (lane 1, 6), or lacking CtpA (lane 2, 7), YvjB (lane 3, 8), ClpP (lane 4, 9), or SsrA (lane 5, 10). Culture supernatants of cells entering the stationary phase were collected, concentrated by TCA precipitation, analyzed by SDS-PAGE and Western blotting with anti-hIL-3 antibody (lane 1-5) or anti-Bs-SsrA tag antibody (lane 6-10). The straight arrows (→) mark SsrA-tagged hIL-3 (lanes 1-4 and lanes 6-9), and run-off translation product (lane 5 and possibly (see text) also in lanes 1-4). Degradation products of (SsrA-tagged) hIL-3 are indicated by ~→.

FIG. 6.

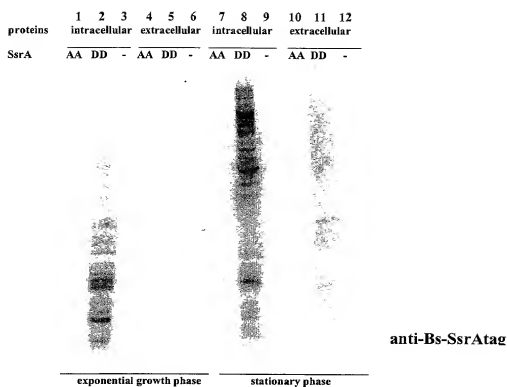


FIG. 6. Tagging of native *B. subtilis* proteins. Total intracellular or extracellular proteins produced by cells in the exponential growth phase or stationary phase of *B. subtilis* 168 expressing wild-type SsrA (AA), 168 *lssrA*^{DD} expressing SsrA^{DD} (DD), or 168 Δ *ssrA* containing no SsrA RNA (-) were analyzed by Western blotting using anti-Bs-SsrAtag antibody.